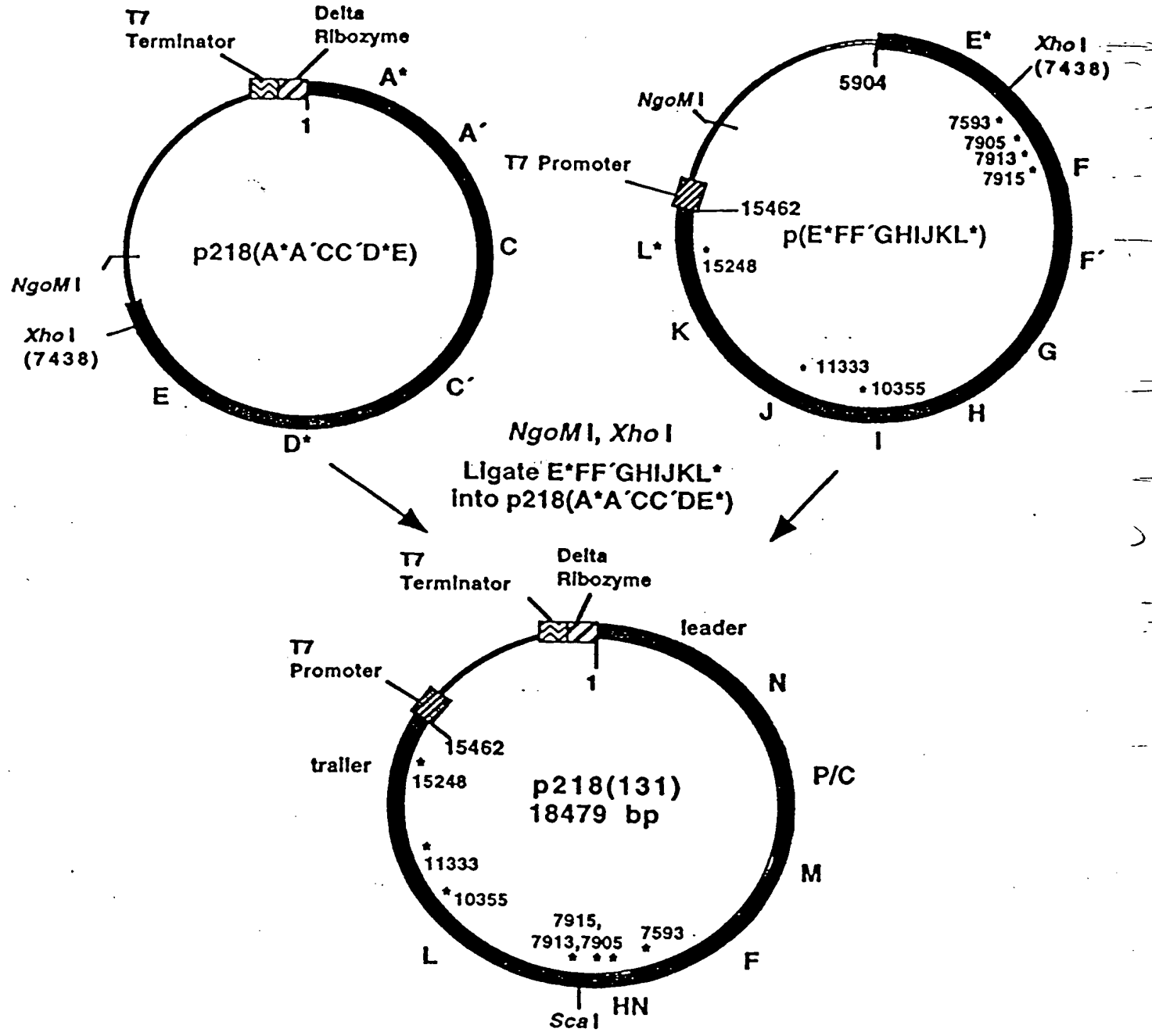
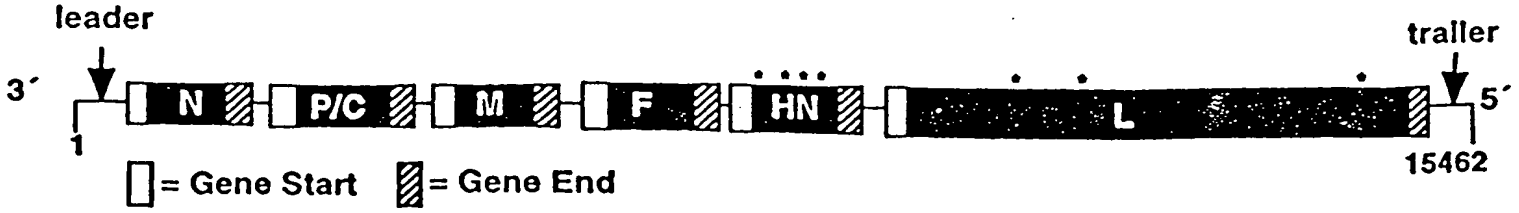
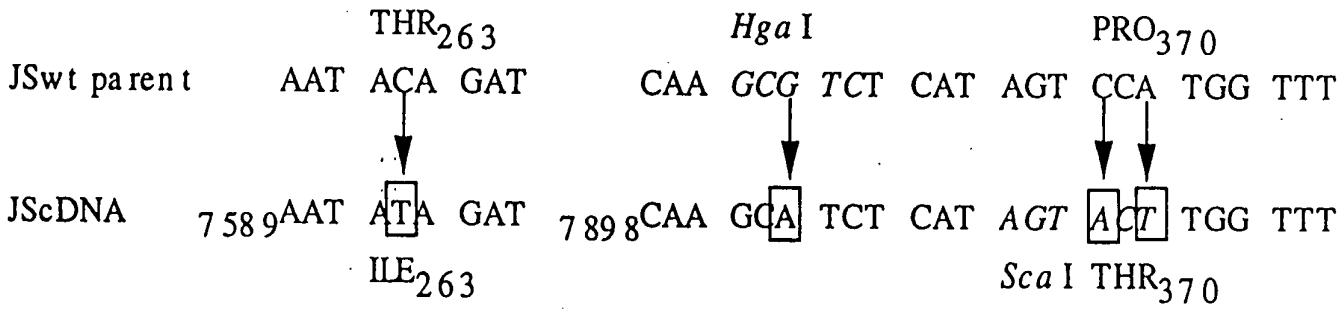



FIG. 1

T7 RNA Polymerase



1) **Introduced and Incidental Mutations in HN gene of JS cDNA [p218(131)]**




2) **Incidental mutations in the L gene of JScDNA [p218(131)]**

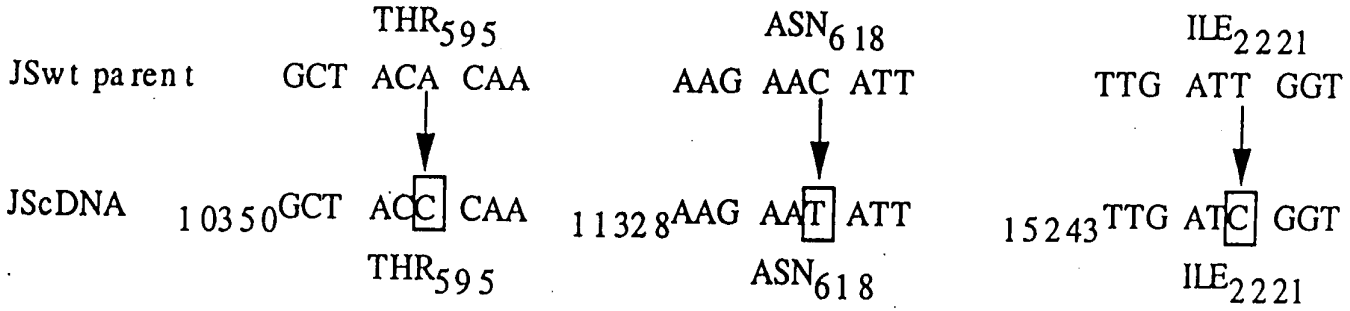



FIG. 2

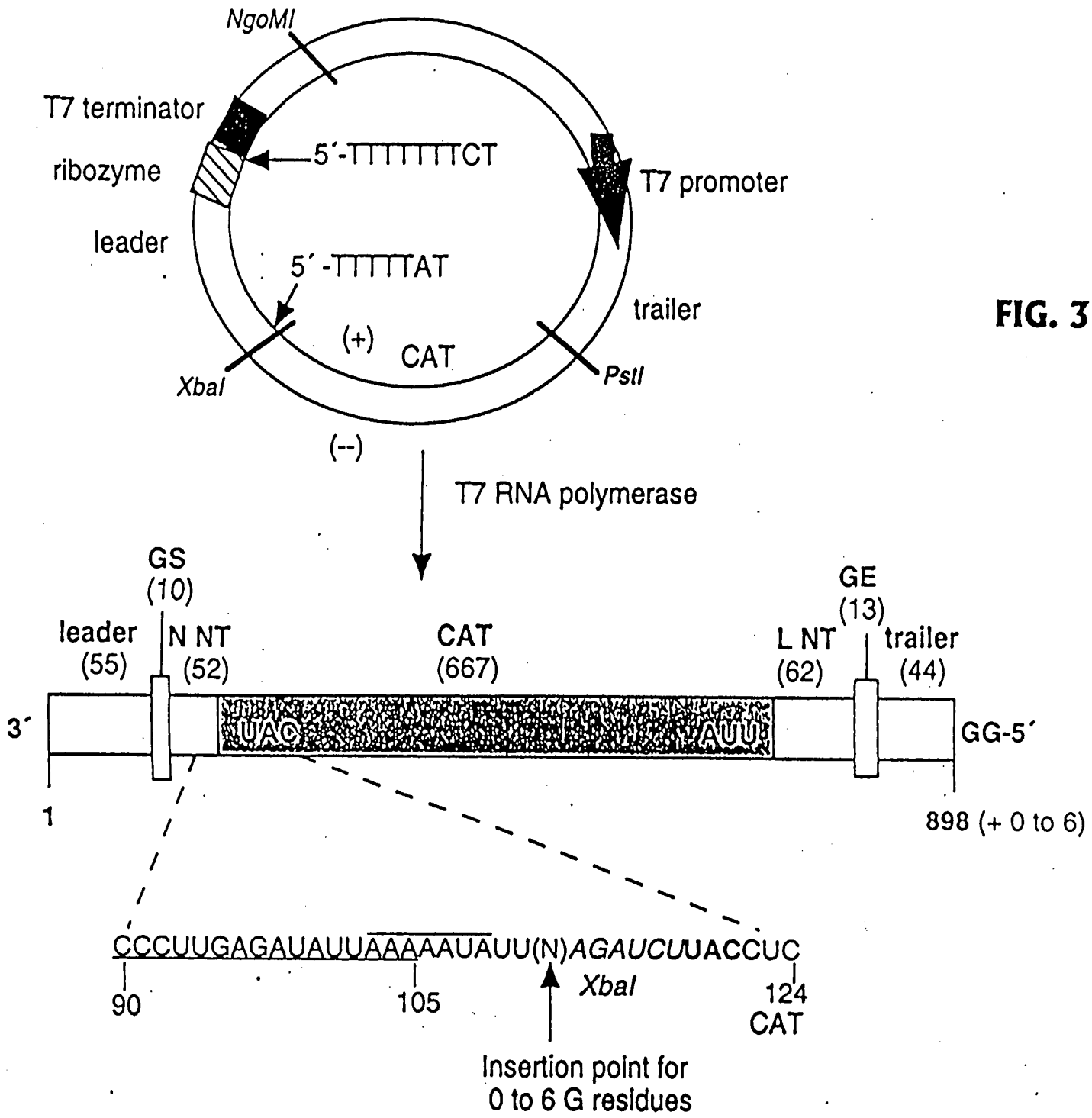

NgoMI
T7 terminator
ribozyme
leader
5′-TTTTTTCT
5′-TTTTTAT
T7 promoter
trailer
(+)
CAT
XbaI
PstI
(--)
T7 RNA polymerase
GS
(10)
leader
(55)
N NT
(52)
CAT
(667)
GE
(13)
L NT
(62)
trailer
(44)
3′
UAC
AUU
GG-5′
1
898 (+ 0 to 6)
CCCUUGAGAUAUUAAAAAUAUU(N)AGAUCUUACCUC
90
105
XbaI
124
CAT
Insertion point for
0 to 6 G residues

**FIG. 3**

FIG. 4

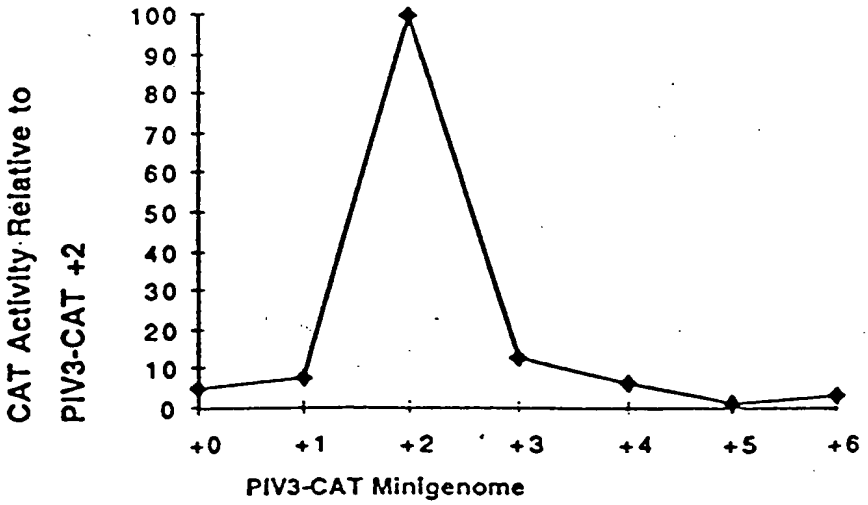

CAT Activity Relative to PIV3-CAT +2
100
90
80
70
60
50
40
30
20
10
0
+0
+1
+2
+3
+4
+5
+6
PIV3-CAT Minigenome

FIG. 5A

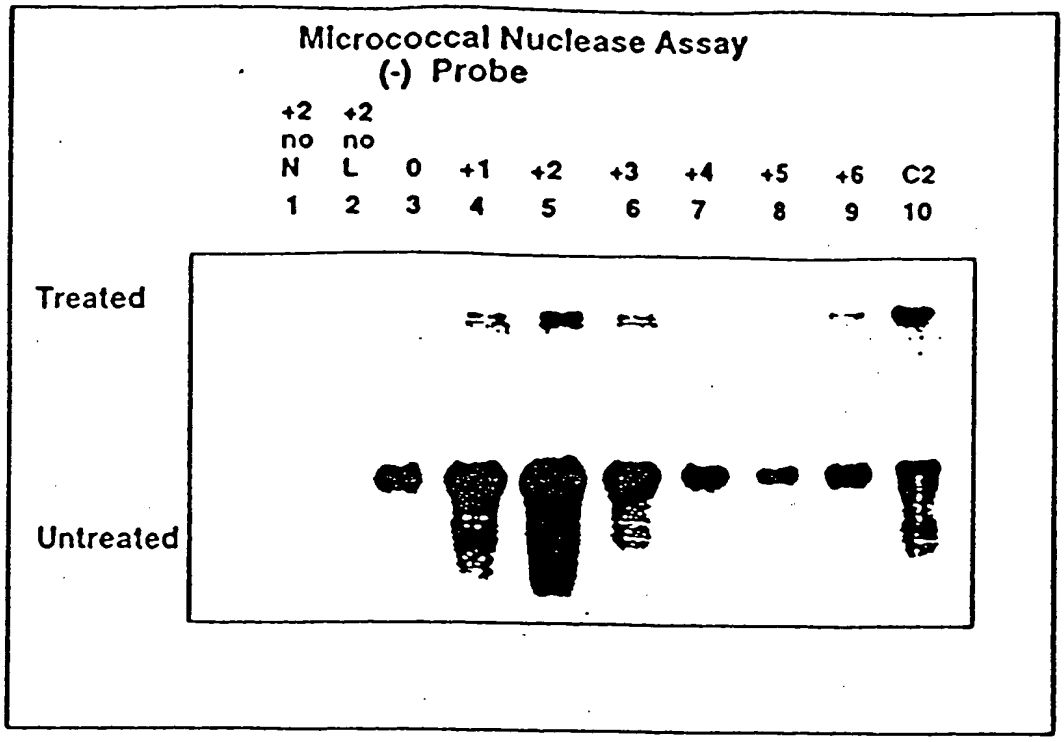

Micrococcal Nuclease Assay
(-) Probe
+2 no N 1
+2 no L 2
0 3
+1 4
+2 5
+3 6
+4 7
+5 8
+6 9
C2 10
Treated
Untreated

FIG. 5B

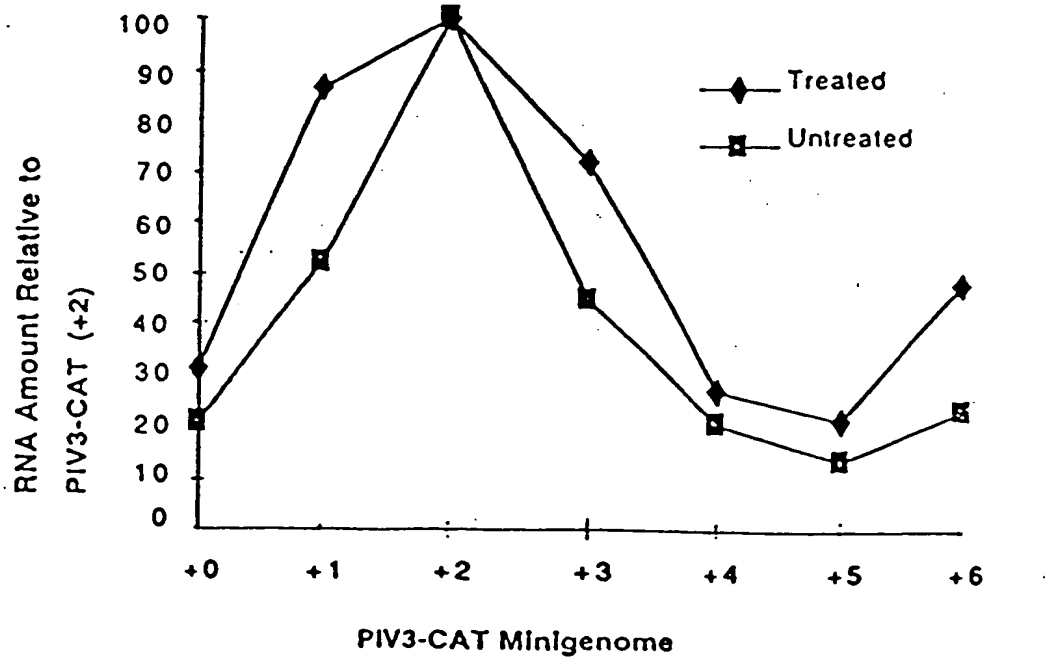

RNA Amount Relative to PIV3-CAT (+2)
100
90
80
70
60
50
40
30
20
10
0
+0
+1
+2
+3
+4
+5
+6
PIV3-CAT Minigenome
Treated
Untreated

FIG. 6A

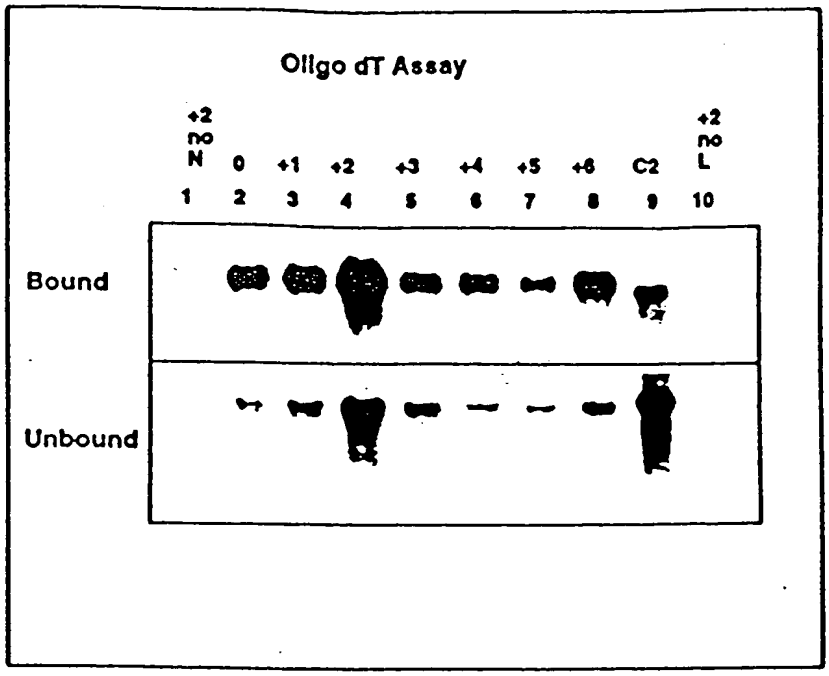

Oligo dT Assay
+2 no N
0
+1
+2
+3
+4
+5
+6
C2
+2 no L
1
2
3
4
5
6
7
8
9
10
Bound
Unbound

FIG. 6B

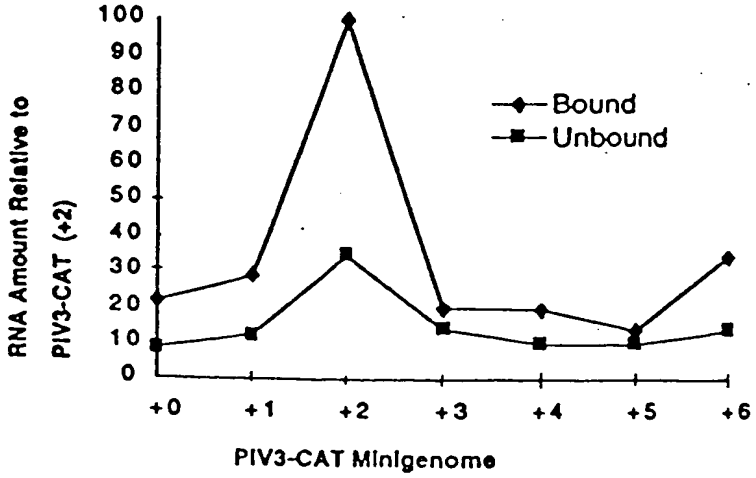

RNA Amount Relative to PIV3-CAT (+2)
100
90
80
70
60
50
40
30
20
10
0
Bound
Unbound
+0
+1
+2
+3
+4
+5
+6
PIV3-CAT Minigenome

FIG. 7A

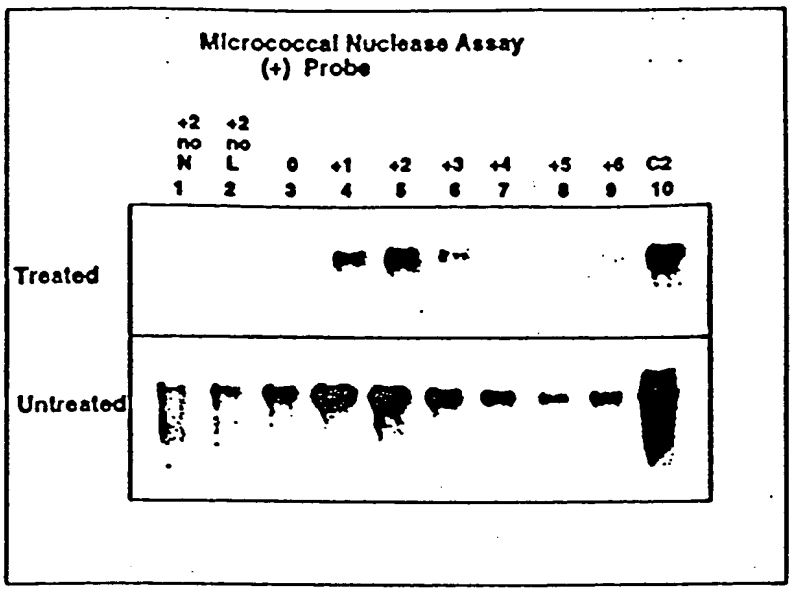
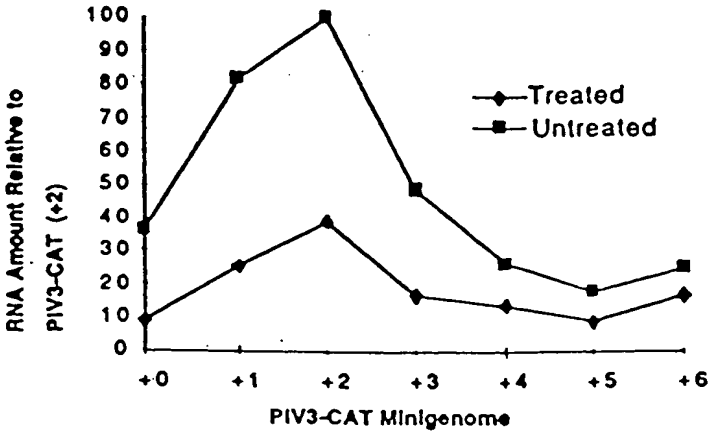

Micrococcal Nuclease Assay
(+) Probe
+2 no N
+2 no L
0
+1
+2
+3
+4
+5
+6
C2
1
2
3
4
5
6
7
8
9
10
Treated
Untreated

FIG. 7B


RNA Amount Relative to PIV3-CAT (+2)
100
90
80
70
60
50
40
30
20
10
0
+0
+1
+2
+3
+4
+5
+6
PIV3-CAT Minigenome
Treated
Untreated

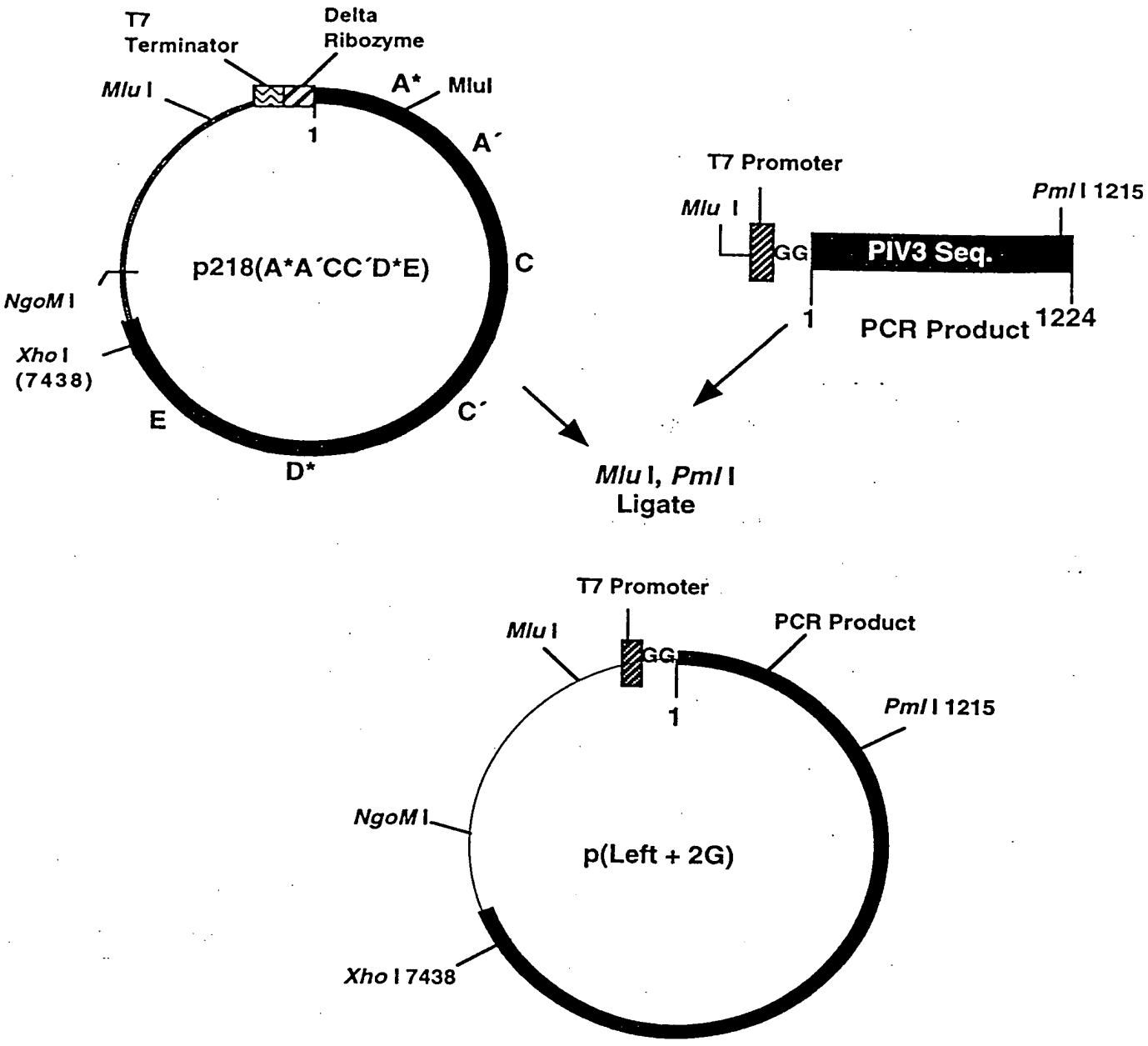

T7
Terminator
Delta
Ribozyme
MluI
A*
MluI
A´
C
C´
E
D*
NgoMI
XhoI
(7438)
1
p218(A*A´CC´D*E)
T7 Promoter
MluI
GG
PIV3 Seq.
PmlI 1215
1
PCR Product
1224
MluI, PmlI
Ligate
T7 Promoter
MluI
GG
PCR Product
PmlI 1215
1
NgoMI
p(Left + 2G)
XhoI 7438

FIG. 8

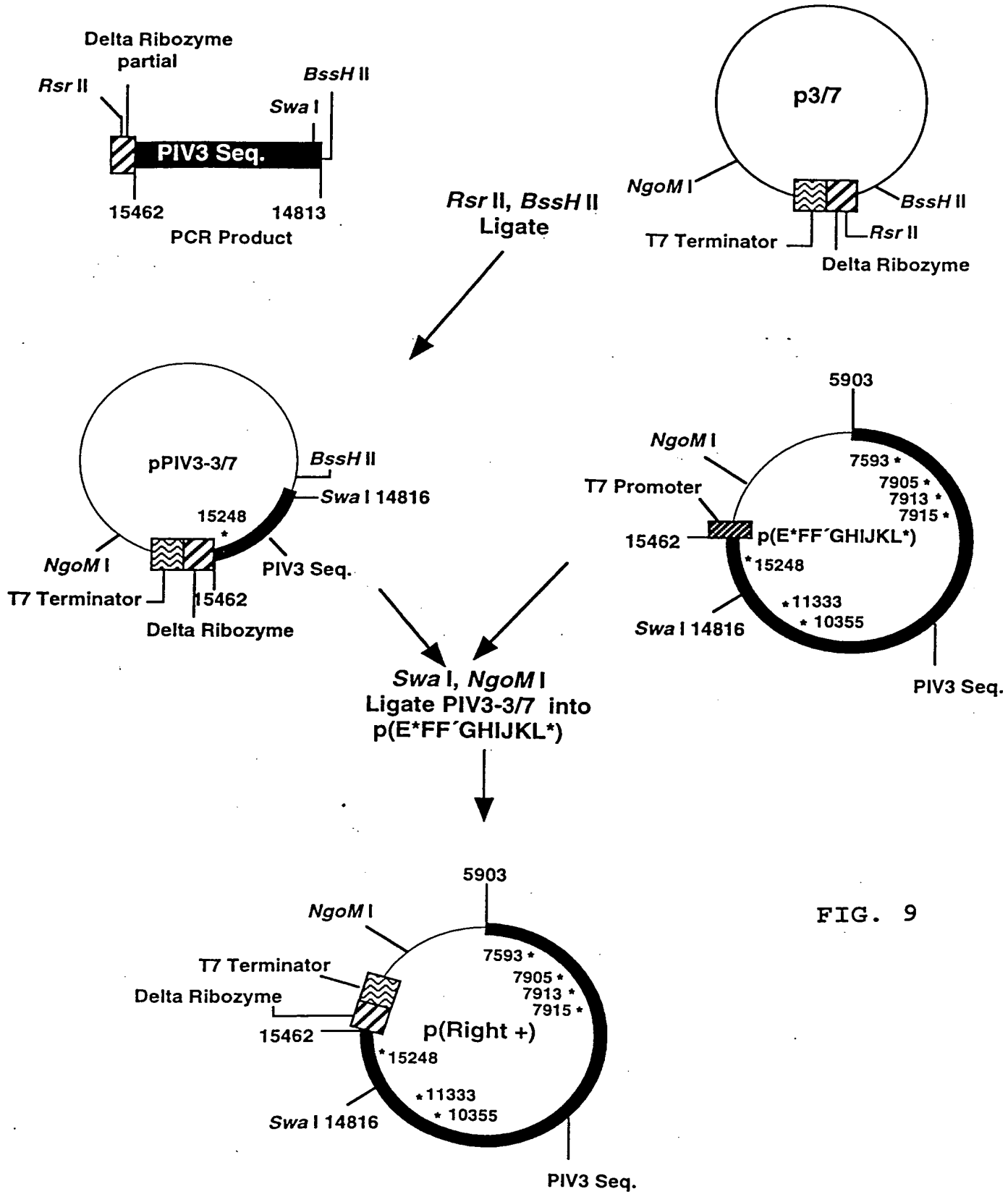
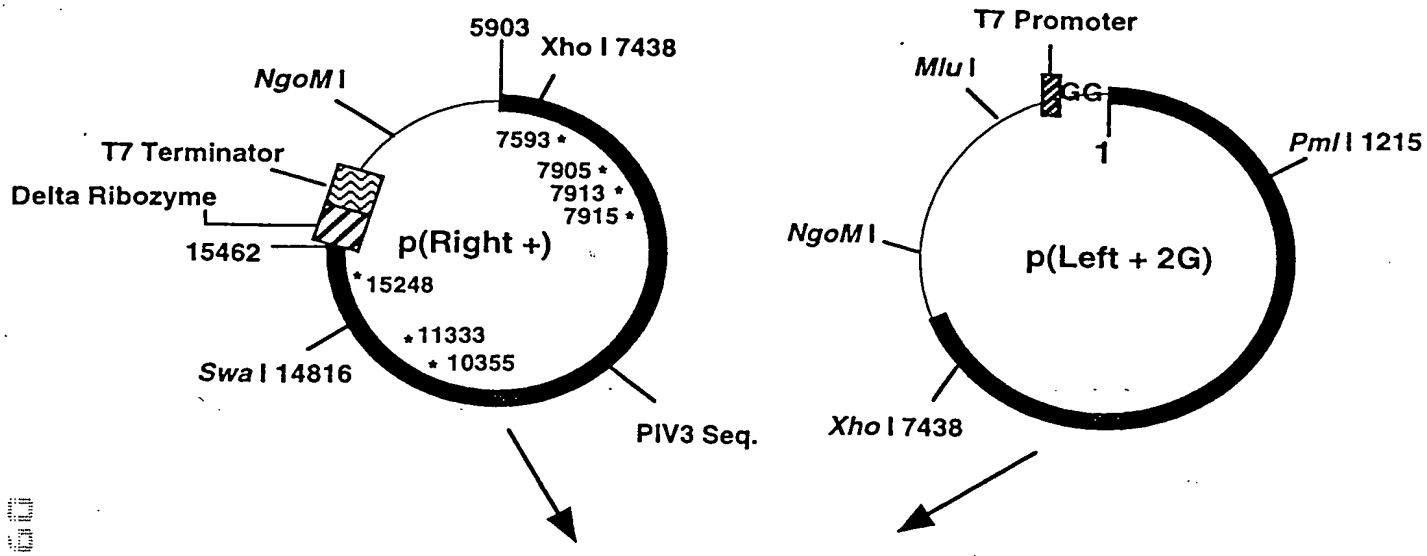
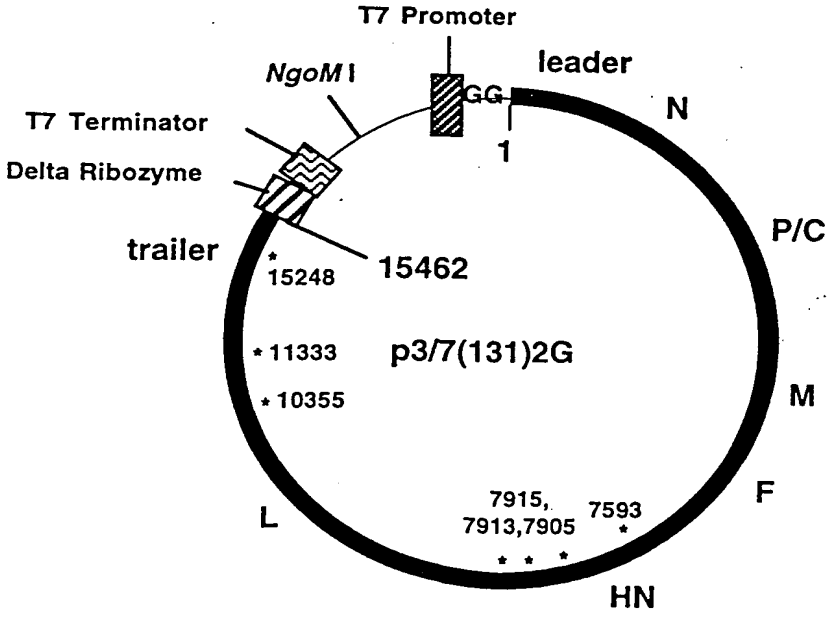

Delta Ribozyme
partial
Rsr II
BssH II
Swa I
PIV3 Seq.
15462
14813
PCR Product
Rsr II, BssH II
Ligate
p3/7
NgoM I
BssH II
T7 Terminator
Rsr II
Delta Ribozyme
pPIV3-3/7
BssH II
Swa I 14816
15248
NgoM I
PIV3 Seq.
T7 Terminator
15462
Delta Ribozyme
5903
NgoM I
7593 *
7905 *
7913 *
7915 *
T7 Promoter
15462
p(E*FF´GHIJKL*)
*15248
*11333
* 10355
Swa I 14816
PIV3 Seq.
Swa I, NgoM I
Ligate PIV3-3/7 into
p(E*FF´GHIJKL*)
5903
NgoM I
7593 *
7905 *
7913 *
7915 *
T7 Terminator
Delta Ribozyme
15462
p(Right +)
*15248
*11333
* 10355
Swa I 14816
PIV3 Seq.

FIG. 9




*NgoM* I, *Xho* I
Ligate (E*FF´GHIJKL*)
into p(Left + 2G)

FIG. 10



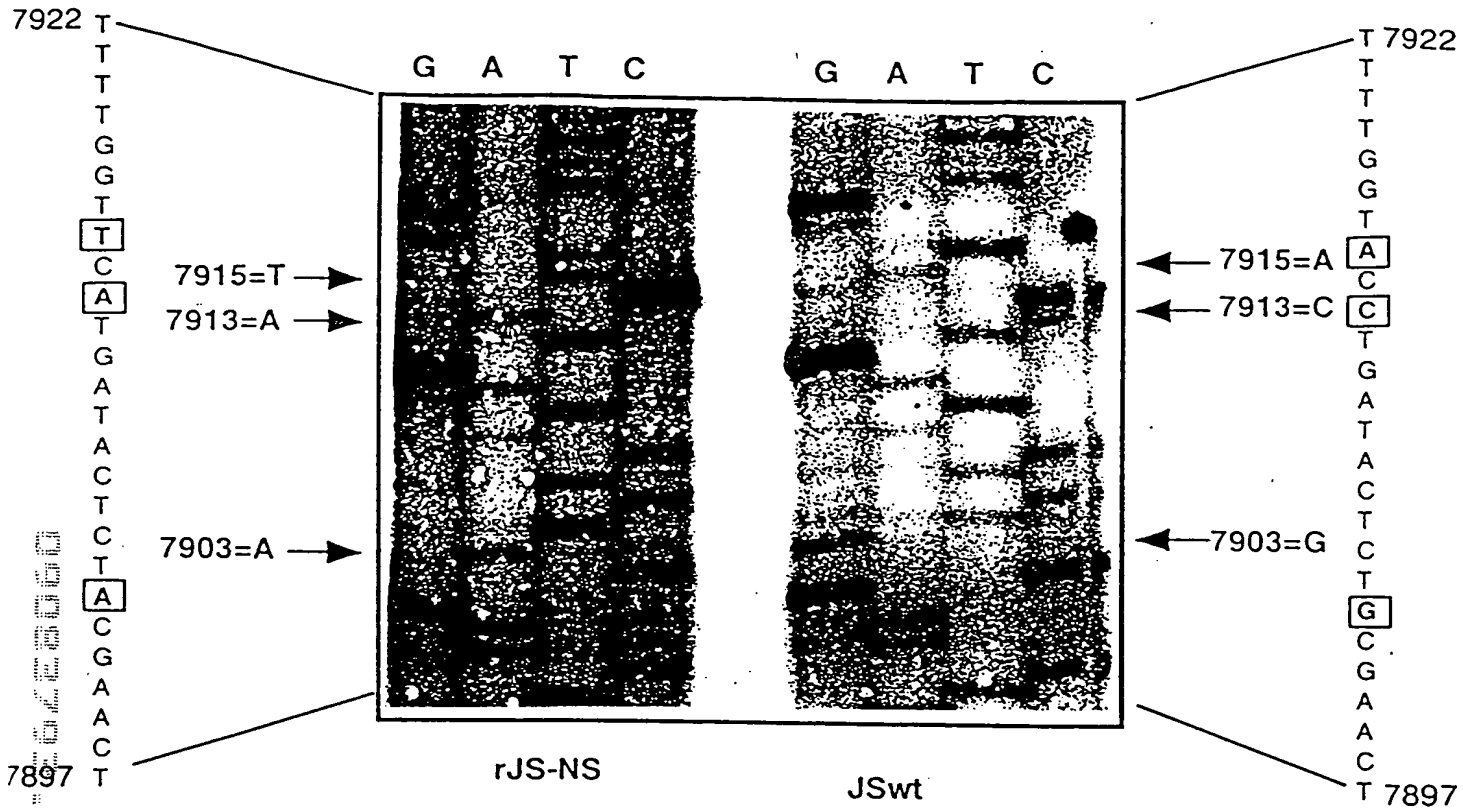

7922
T T T T G G T T C A T G A T A C T C T A C G A A C T
7897
G A T C
G A T C
7915=T
7913=A
7903=A
rJS-NS
JSwt
7915=A
7913=C
7903=G
T 7922
T T T G G T A C C T G A T A C T C T G C G A A C T 7897

FIG. 11

Fig. 12

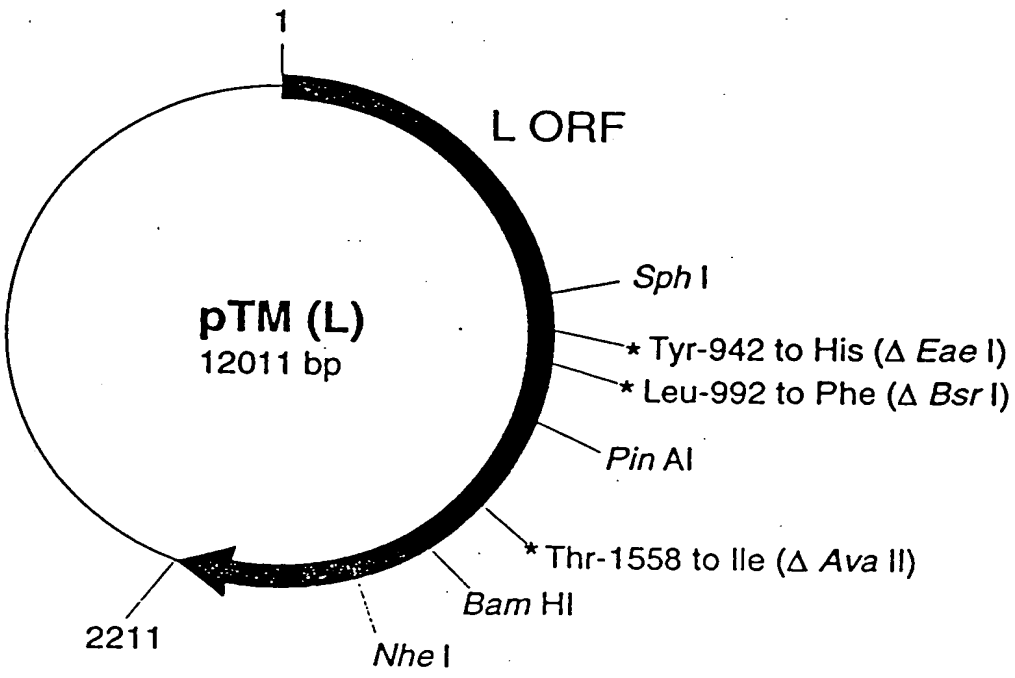

1
L ORF
pTM (L)
12011 bp
Sph I
* Tyr-942 to His (Δ Eae I)
* Leu-992 to Phe (Δ Bsr I)
Pin AI
* Thr-1558 to Ile (Δ Ava II)
Bam HI
Nhe I
2211

FIG. 13

# *cp*45 mutations introduced into rPIV3

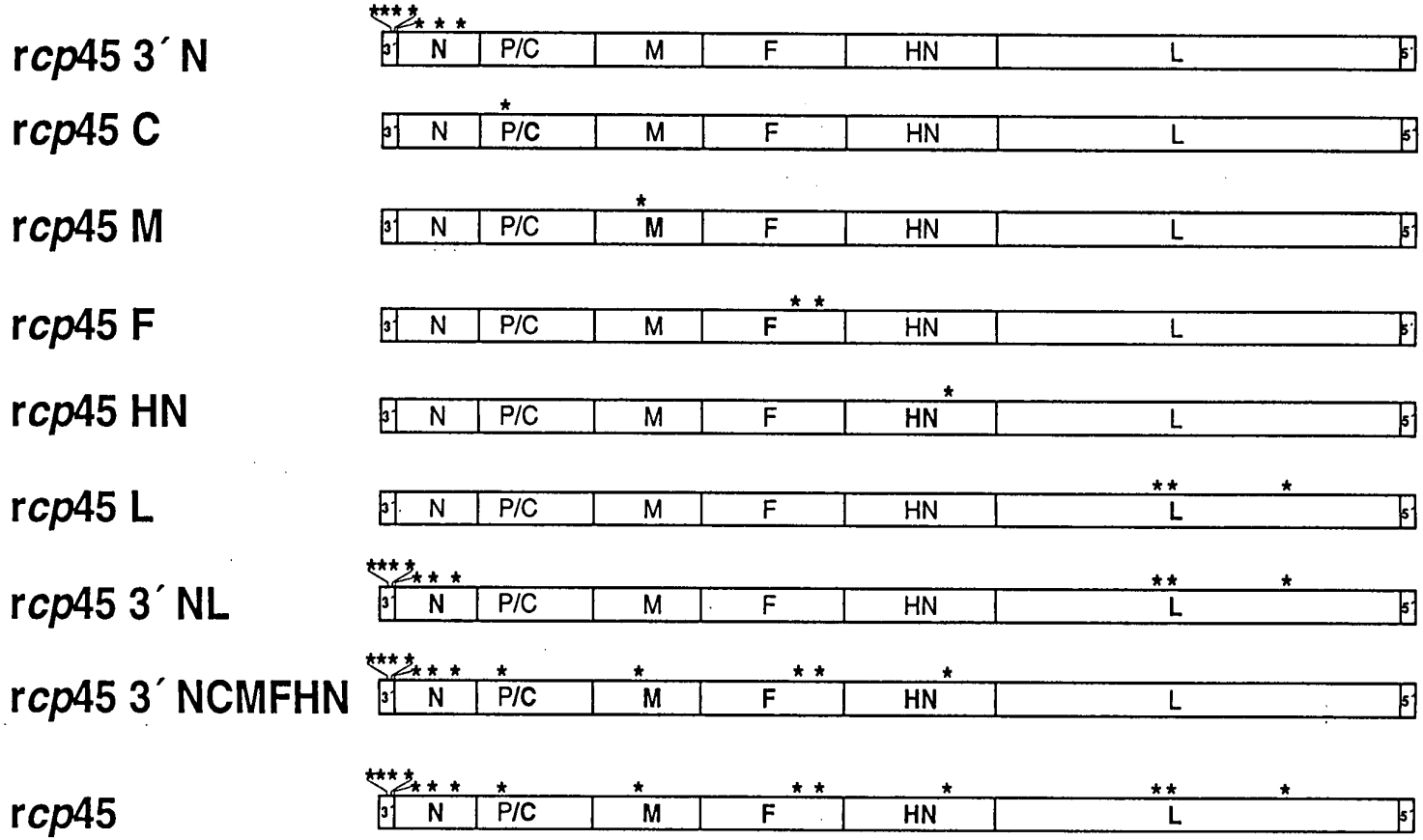



Figure 1: Schematic representation of recombinant PIV3 viruses bearing mutations (*) in the 3´ leader, N gene start, N, C, M, F, HN and/or L proteins.

09093793 : 052295

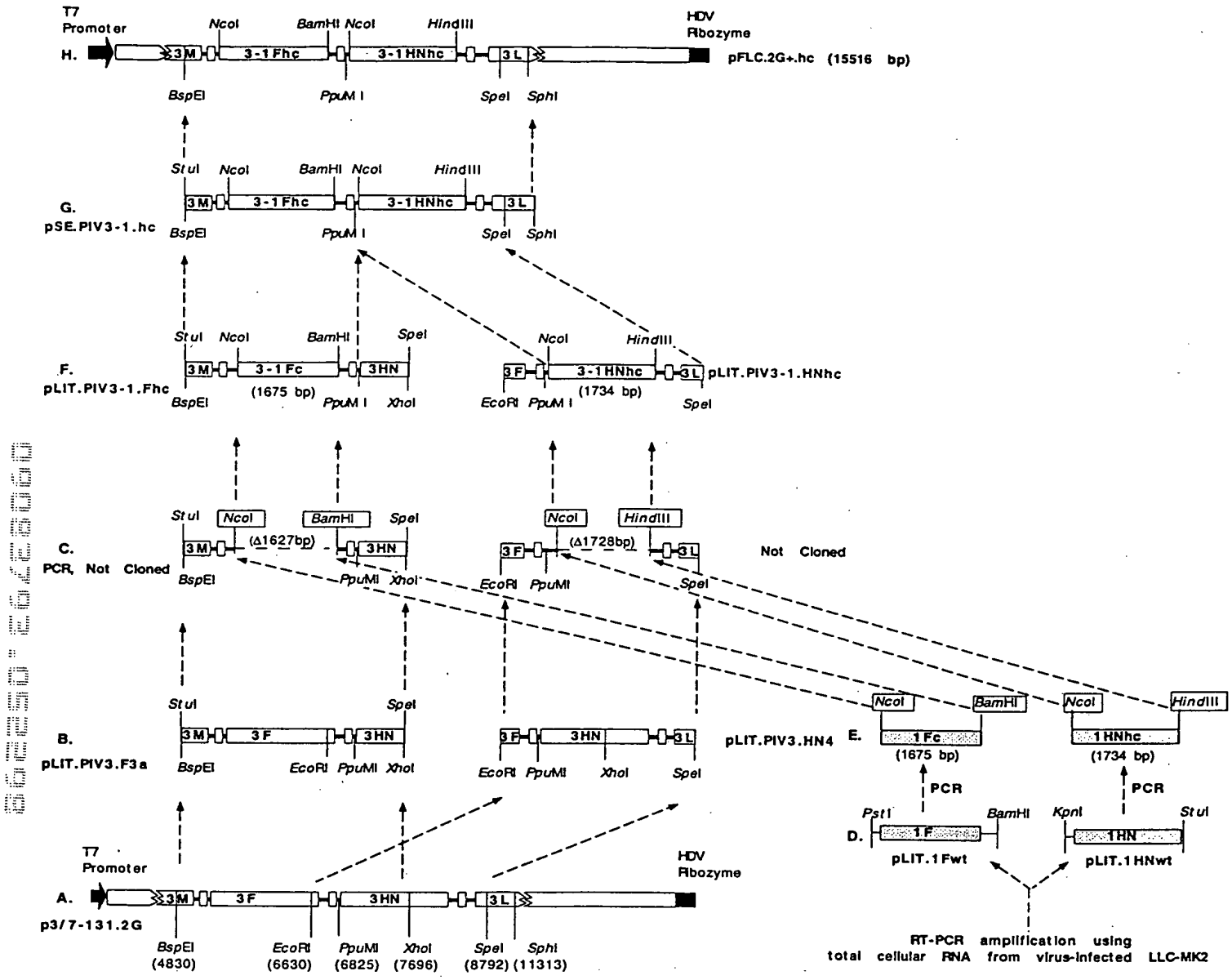

T7 Promoter
HDV Ribozyme
H.
pFLC.2G+.hc (15516 bp)
3M
3-1Fhc
3-1HNhc
3L
NcoI
BamHI
HindIII
BspEI
PpuM I
SpeI
SphI
G.
pSE.PIV3-1.hc
StuI
F.
pLIT.PIV3-1.Fhc
3F
3HN
3-1Fc
3-1HNhc
(1675 bp)
(1734 bp)
XhoI
EcoRI
pLIT.PIV3-1.HNhc
C.
PCR, Not Cloned
(Δ1627bp)
(Δ1728bp)
Not Cloned
B.
pLIT.PIV3.F3a
pLIT.PIV3.HN4
E.
1Fc
1HNhc
PCR
D.
1F
1HN
PstI
KpnI
pLIT.1Fwt
pLIT.1HNwt
A.
p3/7-131.2G
(4830)
(6630)
(6825)
(7696)
(8792)
(11313)
RT-PCR amplification using total cellular RNA from virus-infected LLC-MK2

FIG. 14

86250 2627060

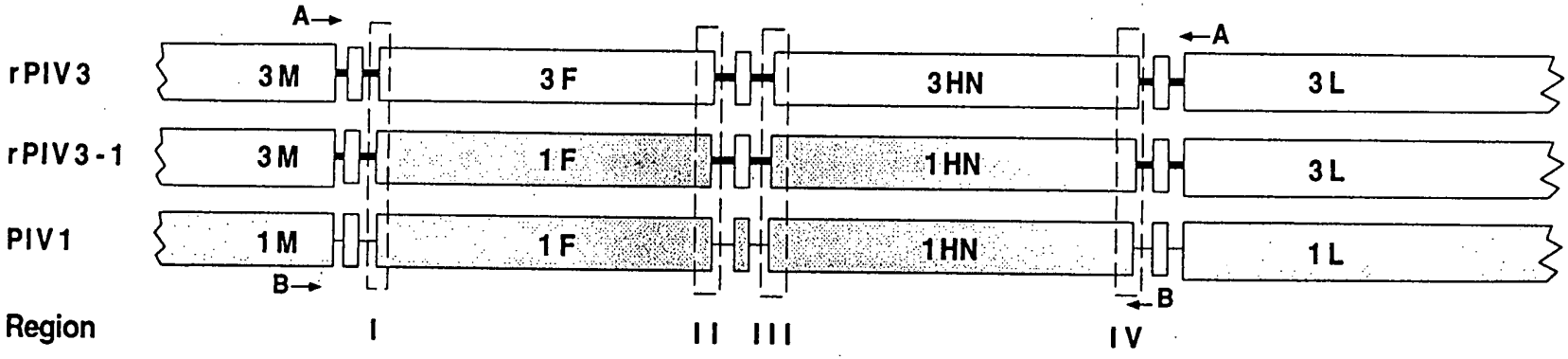



FIG. 15A

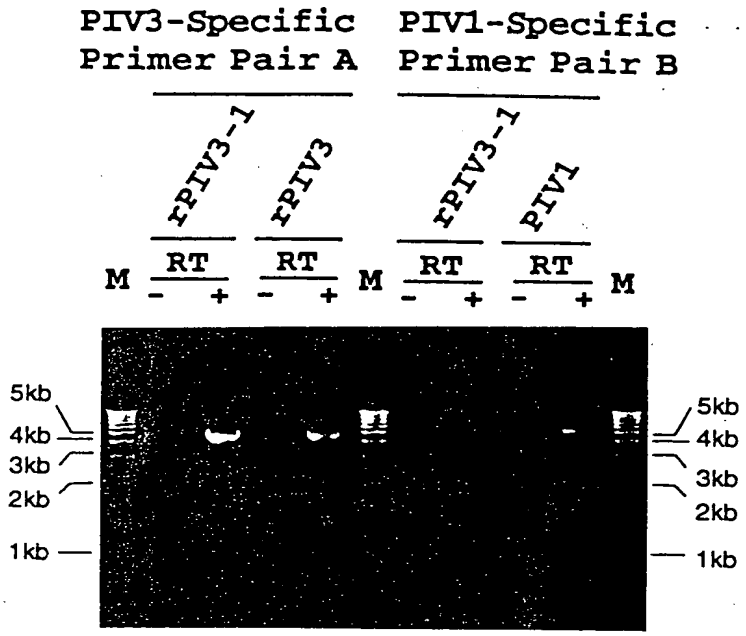
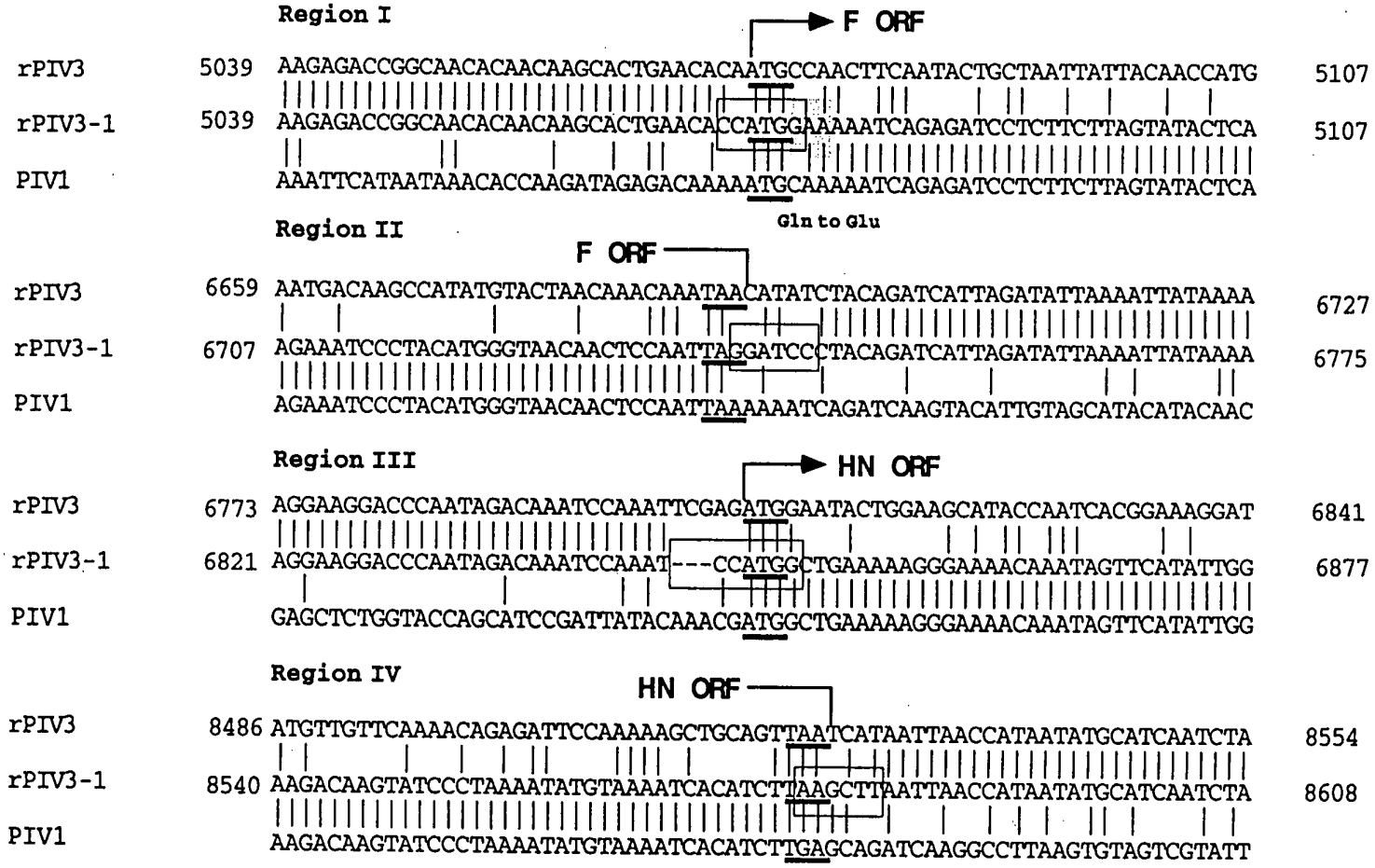

PIV3-Specific Primer Pair A
PIV1-Specific Primer Pair B
rPIV3-1
rPIV3
rPIV3-1
PIV1
M
RT
- +
- +
M
- +
- +
M
5kb
4kb
3kb
2kb
1kb
5kb
4kb
3kb
2kb
1kb

FIG. 15B

862250 26280

# FIG. 16



86250 26260

FIG. 17

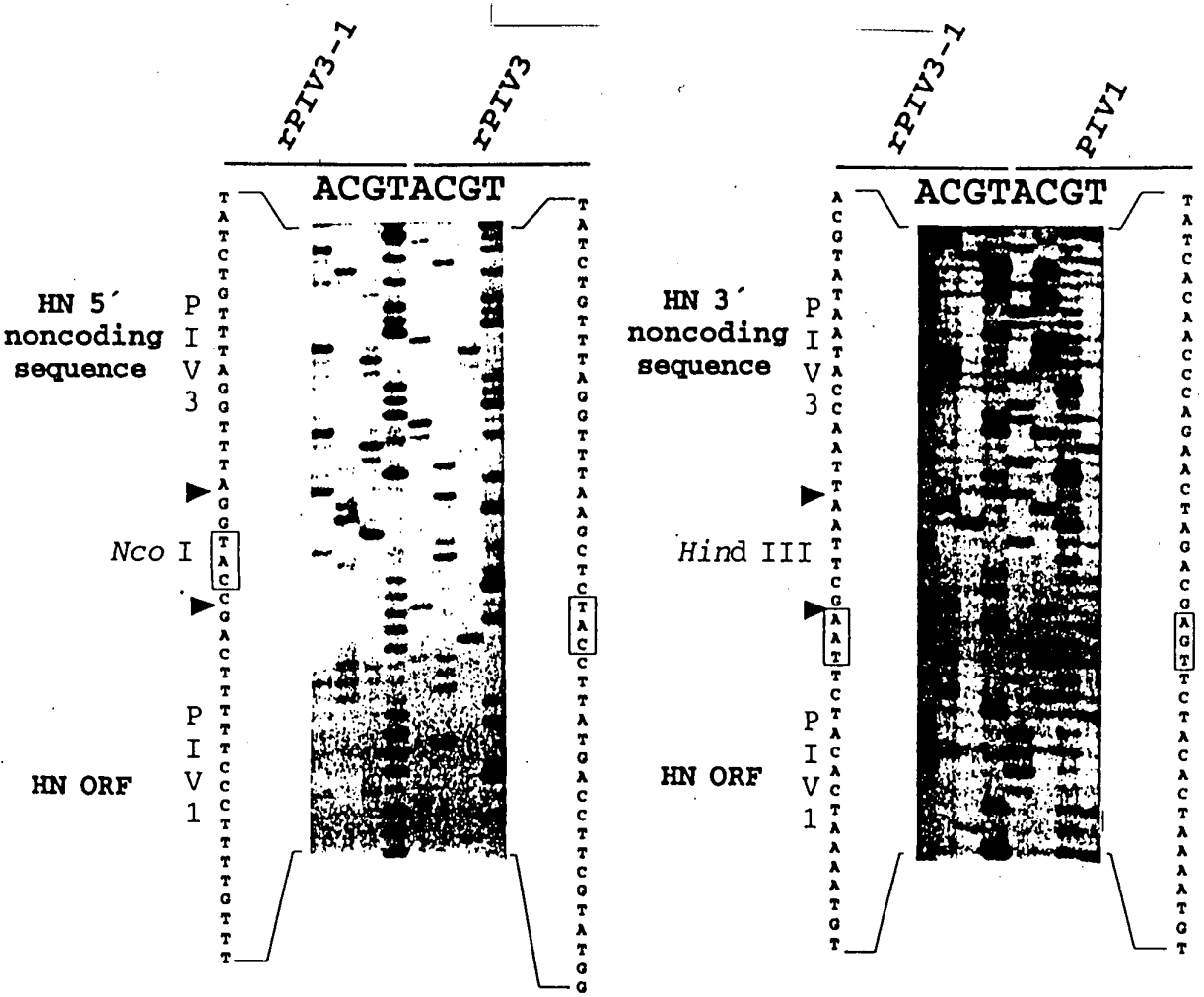
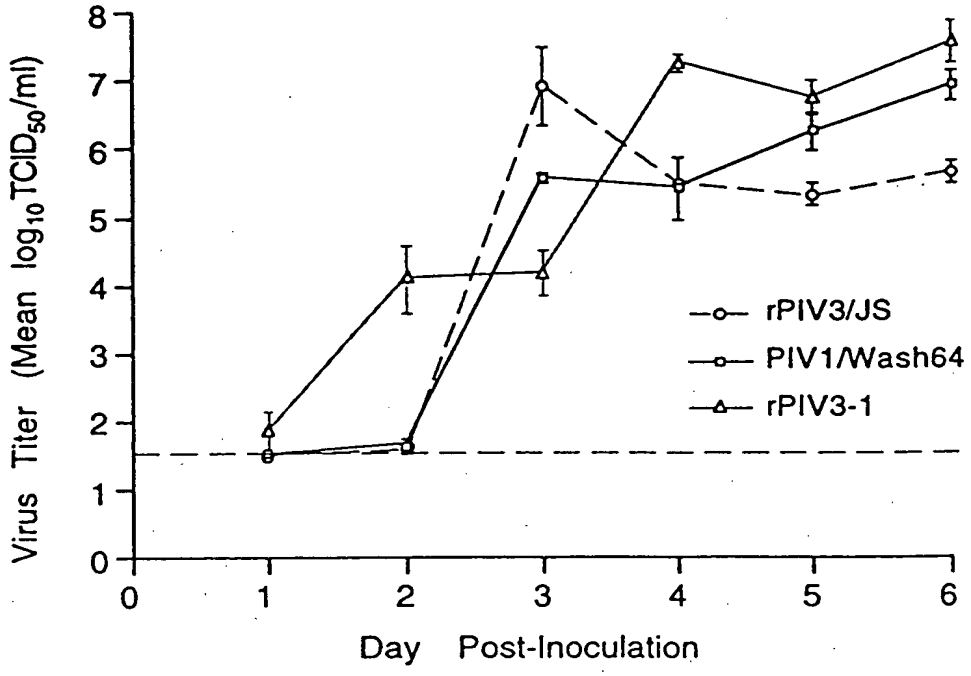


FIG. 18


Virus Titer (Mean log10TCID50/ml)
0
1
2
3
4
5
6
7
8
Day Post-Inoculation
rPIV3/JS
PIV1/Wash64
rPIV3-1

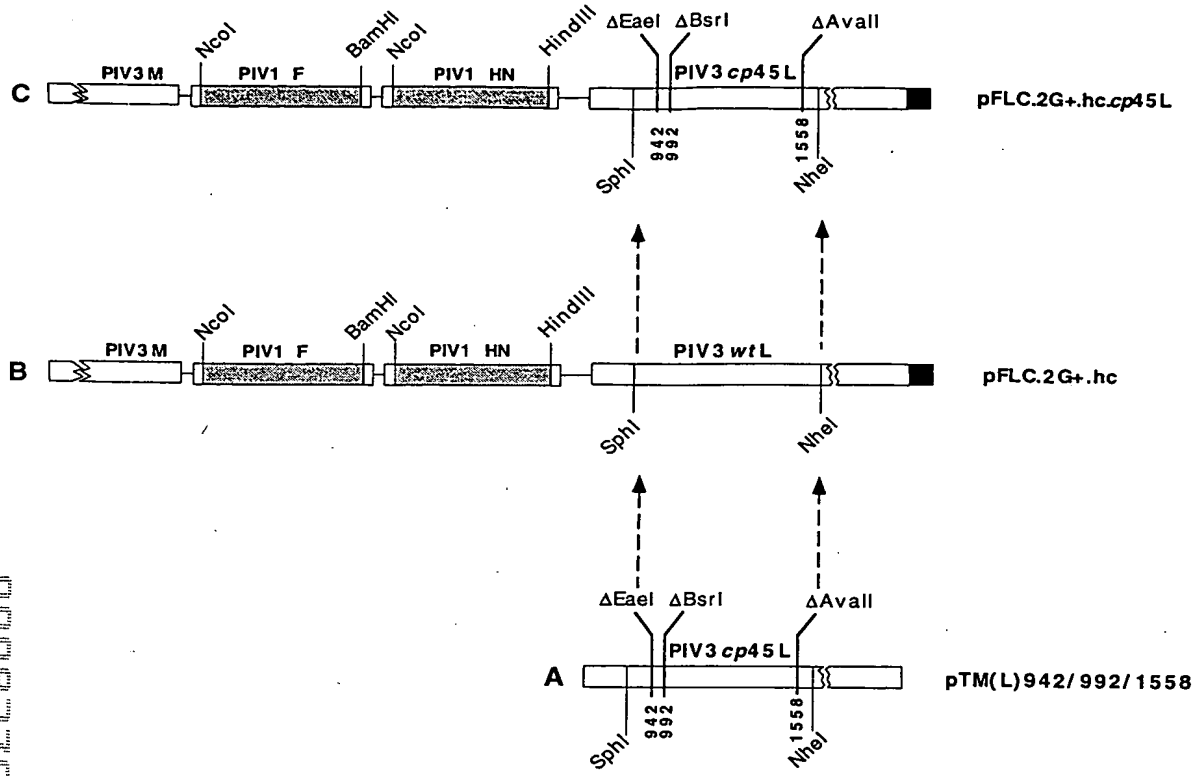

C
PIV3 M
NcoI
PIV1 F
BamHI
NcoI
PIV1 HN
HindIII
ΔEaeI
ΔBsrI
PIV3 cp45 L
ΔAvaII
SphI
942
992
1558
NheI
pFLC.2G+.hc.cp45L
B
PIV3 M
NcoI
PIV1 F
BamHI
NcoI
PIV1 HN
HindIII
PIV3 wt L
SphI
NheI
pFLC.2G+.hc
A
ΔEaeI
ΔBsrI
ΔAvaII
PIV3 cp45 L
SphI
942
992
1558
NheI
pTM(L)942/992/1558

FIG. 19